High-density peptide microarray exploration of the antibody response in a rabbit immunized with a neurotoxic venom fraction

Mikael Engmark[a,b], Martin C. Jespersen[a], Bruno Lomonte[c], Ole Lund[a], Andreas H. Lausten[b].

a. Department of Bio and Health Informatics, Technical University of Denmark, Kgs. Lyngby, Denmark
b. Department of Biotechnology and Biomedicine, Technical University of Denmark, Kgs, Lyngby, Denmark
c. Instituto Coldomiro Picado, Facultad de Microbiologia, Universidad de Costa Rica, San José, Costa Rica

Abstract

Polyvalent snakebite antivenoms derive their therapeutic success from the ability of their antibodies to neutralize venom toxins across multiple snake species. This ability results from a production process involving immunization of large mammals with a broad suite of toxins present in venoms. As a result of immunization with this wide range of toxins, many polyvalent antivenoms have a high degree of cross-reactivity to similar toxins in other snake venoms – a cross-reactivity which cannot easily be deconvoluted. As a proof of concept, we aimed at exploring the opposite scenario by performing a high-throughput evaluation of the extent of cross-reactivity of a polyclonal mixture of antibodies that was raised against only a single snake venom fraction. For this purpose, a venom fraction containing short neurotoxin 1 (SN-1; Uniprot accession number P01416, three-finger toxin (3FTx) family), which is the medically most important toxin from the notorious black mamba (*Dendroaspis polylepis*), was employed. Following immunization of a rabbit, a specific polyclonal antibody response was confirmed by ELISA and immunodiffusion. Subsequently, these antibodies were investigated by high-density peptide microarray to reveal linear elements of recognized epitopes across 742 3FTxs and 10 dendrotoxins. This exploratory study demonstrates in a single immunized animal that cross-reactivity between toxins of high similarity may be difficult to obtain when immunizing with a single 3FTx containing venom fraction. Additionally, this study explored the influence of employing different lengths of peptides in high-density peptide microarray experiments for identification of toxin epitopes. Using 8-mer, 12-mer, and 15-mer peptides, a single linear epitope element was identified in SN-1 with high precision.

Full text:

https://doi.org/10.1016/j.toxicon.2017.08.028